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L3: Entry 5 of 5 File: USPT Dec 26, 2000

US-PAT-NO: 6165471
DOCUMENT-IDENTIFIER: US 6165471 A

TITLE: Homogeneous human papillomavirus capsomere containing compositions, methods for manufacture, and use thereof as diagnostic, prophylactic or therapeutic agents

DATE-ISSUED: December 26, 2000

INVENTOR-INFORMATION:

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US-CL-CURRENT: 424/186.1; 424/192.1, 424/199.1, 435/235.1, 435/320.1, 435/5, 435/69.1, 435/69.3, 536/23.4, 536/23.72

CLAIMS:

What is claimed is:

1. Stable human papillomavirus (HPV) capsomeres which

(i) have a reduced capacity to assemble into virus-like particles (VLPs) relative to a corresponding non-modified HPV L1 protein, wherein reduced capacity means that said capsomeres assemble into VLPs less than 50% relative to a corresponding non-modified HPV L1 protein;

(ii) present at least one virus-neutralizing conformational epitope of the major capsid protein (L1) expressed by a native (wild-type infectious) HPV virus; and

(iii) induce the production of HPV neutralizing antibodies.

2. The stable HPV capsomeres of claim 1, which are selected from the group consisting of HPV-6, HPV-11, HPV-16, HPV-18, HPV-30, HPV-31, HPV-33, HPV-35, HPV-39, HPV-42, HPV-43, HPV-44, HPV-45, HPV-51, HPV-52, HPV-54, HPV-55, HPV-56 and HPV-70 capsomers.

3. The stable capsomeres of claim 1, wherein said stable HPV capsomeres are HPV-11 capsomeres.

4. The stable capsomeres of claim 1, wherein said stable HPV capsomeres are produced by expression of a modified HPV L1 DNA which comprises a carboxyl-

terminal deletion which inhibits or prevents VLP assembly.

5. The stable capsomeres of claim 4, wherein said carboxyl-terminal deletion results in the deletion of at least 30 amino acids of the carboxyl-terminal portion of the L1 protein.

6. The stable capsomeres of claim 5, wherein said modified HPV L1 DNA upon expression results in an L1 protein lacking from about 30 amino acids to about 86 amino acids of the carboxyl-terminal portion of the L1 protein.

7. The stable capsomeres of claim 4, wherein said HPV L1 DNA further comprises at least one addition, substitution or deletion modification which inhibits or prevents disulfide bond formulation.

8. The stable capsomeres of claim 7, wherein the modification comprises the deletion and/or substitution of at least one cysteine codon.

9. The stable capsomeres of claim 8, wherein at least one of said cysteine residue(s) is contained in the region of the L1 protein spanning residues 30 to 86, inclusive, relative to the carboxy terminal end of the L1 protein.

10. The stable capsomeres of claim 1, wherein said capsomeres are produced by the expression of an HPV L1 DNA which has been modified to introduce a mutation which inhibits capsomere-capsomere disulfide bond formation.

11. The stable capsomeres of claim 10, wherein said modification comprises the deletion and/or substitution of at least one cysteine codon.

12. The stable capsomeres of claim 1, wherein said HPV capsomeres induce the production of neutralizing antibodies in a human.

13. The stable capsomeres of claim 12, wherein said HPV are HPV-11 capsomeres.

14. A composition for eliciting neutralizing antibodies against a particular HPV type which comprises an amount of the stable HPV capsomeres of claim 1 sufficient to elicit neutralizing antibodies to said HPV upon challenge and a pharmaceutically acceptable carrier.

15. The composition of claim 14, wherein said composition consists essentially of said stable HPV capsomeres.

16. The composition of claim 14, wherein said stable HPV capsomeres are produced by expression of an HPV L1 DNA containing a modification which upon expression results in stable HPV capsomeres that are have a reduced capacity to assemble into virus-like particles, wherein reduced capacity means that said capsomeres assemble into VLPs less than 50% relative to a corresponding non-modified HPV L1 protein.

17. The composition of claim 16, wherein said modification results in the deletion of at least the 30 carboxyl-terminal amino acids of the HPV L1 protein and/or a deletion, addition or substitution modification which inhibits or prevents formation of a disulfide bond involved in VLP assembly.

18. The composition of claim 17, wherein said modification comprises the deletion and/or substitution of at least one cysteine residue.

19. The composition of claim 14, wherein the stable HPV capsomeres contained therein are selected from the group consisting of HPV-6, HPV-11, HPV-16 and HPV-18 capsomeres, or a mixture thereof.

20. The composition of claim 14, wherein said stable HPV capsomeres are produced by trypsin digestion of HPV virus-like particles or capsomeres, or by treatment with a compound that inhibits the oxidation of reactive sulfhydryls.

21. A method of conferring protection to HPV infection in a susceptible host comprising administering a prophylactically effective amount of stable human capsomeres according to claim 1.

22. The method of claim 21, wherein said stable HPV capsomeres are selected from the group consisting of HPV-6, HPV-11, HPV-16, HPV-18, HPV-30, HPV-31, HPV-33, HPV-35, HPV-39, HPV-42, HPV-43, HPV-44, HPV-45, HPV-51, HPV-52, HPV-54, HPV-55, HPV-56, HPV-70 capsomeres, and mixtures thereof.

23. The method of claim 22, wherein said stable HPV capsomeres comprise HPV-11 capsomeres.

24. The method of claim 21, wherein said stable capsomeres contain a carboxyl-terminal deletion which removes at least 30 amino acids of the carboxy-terminal portion of the L1 protein.

25. The method of claim 24, wherein said stable capsomeres lack from 30 to 86 amino acids of said HPV L1 protein.

26. The method of claim 21, wherein said stable HPV capsomeres are produced by expression of an HPV L1 DNA which has been modified to inhibit capsomere-capsomere disulfide bond formation.

27. The method of claim 26, wherein the modification comprises the deletion and/or substitution of at least one cysteine codon.

28. A diagnostic composition comprising a diagnostically effective amount of stable human papillomavirus capsomeres according to claim 1.

29. The diagnostic composition of claim 28, wherein said capsomeres are directly or indirectly attached to a detectable label.

30. An HPV L1 DNA that contains a modification that upon expression it results in the production of stable HPV capsomeres which have a reduced capacity to assembly into virus-like particles relative to a corresponding non-modified HPV L1 protein, wherein reduced capacity means that said capsomeres assembly into VLPs less than 50% relative to a corresponding non-modified HPV L1 protein, and which present at least one virus-neutralizing conformational epitope expressed by a native HPV virion.

31. The HPV L1 DNA of claim 30, which comprises a modification which inhibits or prevents the formation of at least one disulfide bond involved in VLP assembly.

32. The HPV L1 DNA of claim 31, wherein said modification comprises the substitution or deletion of at least one cysteine residue.

33. The HPV L1 DNA of claim 32, which further comprises a carboxyl terminal deletion which inhibits the formation of virus-like particles.

34. The HPV L1 DNA of claim 33, wherein said carboxyl-terminal deletion results in the elimination of at least 30 amino acids of the L1 protein.

35. The HPV L1 DNA of claim 34, wherein said carboxyl-terminal deletion results in the elimination of from about 30 to 86 amino acids of the L1 protein.

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L2: Entry 4 of 4 File: USPT Jul 17, 2001

US-PAT-NO: 6261765
DOCUMENT-IDENTIFIER: US 6261765 B1

TITLE: In vitro method for disassembly/reassembly of papillomavirus virus-like particles (VLPs)

DATE-ISSUED: July 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/5; 435/235.1, 435/236, 435/238, 435/239

CLAIMS:

What is claimed is:

1. A method for producing a homogeneous papillomavirus virus-like particle (VLP) containing composition that comprises the following steps:

(i) contacting a papillomavirus virus-like particle (VLP) containing composition for a sufficient time with a solution comprising a concentration of at least one sulfhydryl reducing agent and having an ionic strength which is sufficient to result in at least 70% of such VLPs disassembling into smaller, correctly-folded L1 protein containing molecules; and

(ii) inducing reassembly of said smaller, correctly-folded molecules into VLPs by the removal or oxidation of the sulfhydryl reducing agent.

2. A homogeneous papillomavirus VLP composition produced according to the method of claim 1.

3. The VLP composition of claim 2, wherein said VLPs are human papillomavirus VLPs.

4. The VLP composition of claim 3, wherein said human papillomavirus VLPs are selected from the group consisting of HPV-6, HPV-11, HPV-16, HPV-18, HPV-30, HPV-31, HPV-33, HPV-35, HPV-39, HPV-41, HPV-42, HPV-43, HPV-44, HPV-45, HPV-52, HPV-54, HPV-55, HPV-56, HPV-70 and mixtures thereof.

5. The method of claim 1, wherein the sulfhydryl reducing agent is oxidized or removed by dialysis, diafiltration or column chromatography.

6. The method of claim 1, wherein the ionic strength is raised during the

reassembly step (ii) to 0.5 M or higher to enhance the stability of the reassembled VLPs.

7. The method according to claim 1, wherein the concentration of reducing agent used in step (i) is at least 1% by weight.

8. The method according to claim 1, wherein said sulfhydryl reducing agent is selected from the group consisting of glutathione, dithiothreitol, .beta.-mercaptoethanol, dithioerythritol, cysteine, hydrogen sulfide and mixtures thereof.

9. The method according to claim 1, wherein the ionic strength of the solution used for disassembly is 0.25 M or less.

10. The method according to claim 9, wherein the ionic strength of the solution used for disassembly is 0.15 M or less.

11. The method according to claim 9, wherein in step (i) the VLPs are contacted with the sulfhydryl reducing agent solution for at least 2 hours.

12. The method according to claim 11, wherein said contacting is effected for at least about 16 hours.

13. The method according to claim 12, wherein contacting is effected for a time ranging from at least 16 to 24 hours.

14. The method according to claim 1, wherein the VLPs are human papillomavirus VLPs.

15. The method according to claim 14, wherein said human papillomavirus VLPs are selected from the group consisting of HPV-6, HPV-1, HPV-16, HPV-18, HPV-30, HPV-31, HPV-33, HPV-35, HPV-39, HPV-41, HPV-42, HPV-43, HPV-44, HPV-45, HPV-52, HPV-54, HPV-55, HPV-56, HPV-70 and mixtures thereof.

16. The method according to claim 1, wherein said VLPs are substantially free of aggregates larger than about >0.45 .mu.m in diameter.

17. The method according to claim 1, which does not include the use of protease(s) and/or chelating agent(s).

18. The method of claim 1, wherein the solution has an ionic strength which is less than 0.5 M.

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L1: Entry 2 of 2 File: USPT Jul 9, 2002

US-PAT-NO: 6416945
DOCUMENT-IDENTIFIER: US 6416945 B1

TITLE: Vitro method for disassembly/reassembly of papillomavirus virus-like particles (VLPS)

DATE-ISSUED: July 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Suzich; JoAnn	Washington Grove	MD		

US-CL-CURRENT: 435/5; 435/235.1, 435/236, 435/239

CLAIMS:

What is claimed is:

1. A method for producing papillomavirus virus-like particles comprised of L1 proteins or a combination of L1 and L2 proteins which have encapsulated therein at least one desired moiety comprising:

(i) disassembling a VLP composition comprising papillomavirus VLPs constituted of L1 proteins or a combination of L1 and L2 proteins by contacting such VLPs for a prolonged time period with a solution containing a high concentration of reducing agent;

(ii) contacting the resultant smaller, correctly-folded components with a solution containing at least one moiety that is to be encapsulated in reassembled papillomavirus VLPs, and optionally purified L2 protein; and

(iii) removing said reducing agent or adding oxidants to provide for reassembly of said smaller, correctly-folded components into papillomavirus VLPs containing said moiety.

2. The method of claim 1, wherein the at least one moiety encapsulated in said reassembled VLPs is selected from the group consisting of nucleic acid sequences, radionuclides, anti-cancer agents, anti-viral agents, cell growth modulating agents, hormones, peptides, cytokines, antigens, toxins and mixtures thereof.

3. The method according to claim 2, wherein said moiety is a DNA.

4. The method according to claim 3, wherein said DNA encodes for a selectable marker.

5. The method according to claim 4, wherein said selectable marker is expressed in cells infected by said papillomavirus.

6. The method according to claim 3, wherein said DNA is at most about 8 kilobases.

7. A method of introducing a moiety into cells normally infected by papillomavirus comprising administering an effective amount of VLPs produced according to claim 1.

8. The method of claim 7, wherein said VLPs contain a DNA, antiviral agent or anti-cancer agent.

9. The method of claim 7, wherein said cells are epithelial cells or non-epithelial cells which are permissive for HPV VLP entry.

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